WO0041648

Publication Title:

APPARATUS AND METHOD FOR STERILIZING, SEEDING, CULTURING, STORING, SHIPPING AND TESTING TISSUE, SYNTHETIC, OR NATIVE VASCULAR GRAFTS

Abstract:

Abstract of WO0041648

An apparatus and method for sterilizing, seeding, culturing, storing, 122b shipping, and testing vascular grafts and other prosthesis is disclosed. Specifically, the present invention relates to an apparatus and method for seeding and culturing vascular grafts with human cells. The apparatus includes a fluid reservoir, a pump, an alternating pressure source, and at least one treatment chamber. In accordance with the present invention, fluid is pumped directly through the vascular graft located within the treatment chamber, subjecting the vascular graft to radial and shear stresses. In addition, the alternating pressure source expands and contracts the treatment chamber, thereby applying a varying axial stress to the scaffold positioned within the treatment chamber. Applying shear, radial, and axial stresses to the vascular graft during seeding and culturing simulates the physiological conditions experienced by the graft once implanted.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61F 2/06 | A1

(11) International Publication Number: WO 00/41648

(43) International Publication Date: 20 July 2000 (20.07.00)

(21) International Application Number: PCT/US00/01003

(22) International Filing Date: 14 January 2000 (14.01.00)

(30) Priority Data:
60/118,656 14 January 1999 (14.01.99) US

(71) Applicant: ADVANCED TISSUE SCIENCES, INC. [US/US]; 10933 North Torrey Pines Road, La Jolla, CA 92037–1005 (US).

(72) Inventors: FINO, Michael, R., Jr.; 11340–1 Portobelo Drive, San Diego, CA 92124 (US). LEE, Ann, A.; 1100 Calle del Cerro #58, San Clemente, CA 92672 (US). BAUMGARTNER, Mark, F.; 7125 Charmant Drive #915, San Diego, CA 92126 (US). KOLLER, Fred, R.; 3646 Torrey View Court, San Diego, CA 92130 (US).

(74) Agents: POISSANT, Brian, M. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

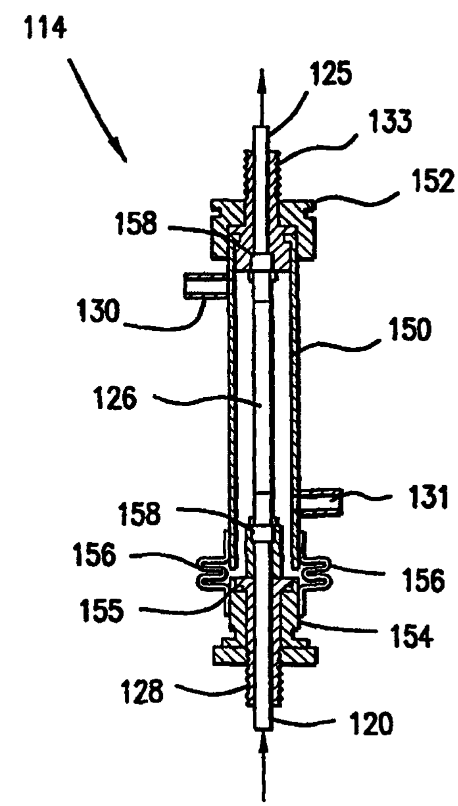
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: APPARATUS AND METHOD FOR STERILIZING, SEEDING, CULTURING, STORING, SHIPPING AND TESTING TISSUE, SYNTHETIC, OR NATIVE VASCULAR GRAFTS

(57) Abstract

An apparatus and method for sterilizing, seeding, culturing, storing, shipping, and testing vascular grafts and other prosthesis is disclosed. Specifically, the present invention relates to an apparatus and method for seeding and culturing vascular grafts with human cells. The apparatus includes a fluid reservoir, a pump, an alternating pressure source, and at least one treatment chamber. In accordance with the present invention, fluid is pumped directly through the vascular graft located within the treatment chamber, subjecting the vascular graft to radial and shear stresses. In addition, the alternating pressure source expands and contracts the treatment chamber, thereby applying a varying axial stress to the scaffold positioned within the treatment chamber. Applying shear, radial, and axial stresses to the vascular graft during seeding and culturing simulates the physiological conditions experienced by the graft once implanted.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

APPARATUS AND METHOD FOR STERILIZING, SEEDING, CULTURING, STORING, SHIPPING AND TESTING TISSUE, SYNTHETIC, OR NATIVE VASCULAR GRAFTS

This application claims priority to the provisional patent application entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping and Testing Tissue, Synthetic, or Native Vascular Grafts", serial no. 60/118,656, filed January 14, 1999.

This invention was made with United States Government support under Copperative Agreement No. 70NANB7H3060 awarded by NIST.

The United States Government has certain rights to the invention.

BACKGROUND OF THE INVENTION

Technical Field

The present invention relates to the sterilization, seeding, culturing, storing, shipping, and testing of vascular grafts and other prosthetic devices. Specifically, the present invention relates to an apparatus and method for sterilizing vascular grafts and then seeding and culturing the grafts with human cells, resulting in grafts that are more likely to display the biochemical, physical, and structural properties of native tissues.

Discussion of the Related Art

Vascular and thoracic surgeons use vascular grafts to repair or replace segments of arterial and venous blood vessels that are weakened, damaged, or obstructed due to trauma or disease, such as aneurysm, atherosclerosis, and diabetes mellitus. Historically, vascular grafts have been either homografts, such as the patient's own saphenous vein or internal mammary artery, prosthetic grafts made of synthetic materials such as polyester (e.g., Dacron), expanded polytetraflouroethylene (ePTFE), and other composite materials, or fresh or fixed biological tissue grafts.

However, synthetic grafts generally have inadequate patency rates for many uses, while the harvesting of homografts requires extensive surgery which is time-consuming, costly, and traumatic to the patient. Fixed tissue grafts do not allow for infiltration and colonization by the host cells, which is essential to remodeling and tissue maintenance. Consequently, fixed tissue grafts degrade with time and will eventually malfunction.

Due to the inadequacies of these currently available synthetic and biological grafts, and the high cost and limited supply of homografts, tissue engineered grafts are being developed which are sterilized, then seeded and cultured, in vitro, with cells. These tissue engineered grafts may be superior to other grafts for use in replacement therapy in that they may display the long term dimensional stability and patency of native arteries and vessels with normal physiologic functionality.

Historically, the seeding and culturing of vascular grafts, and tissue in general, has taken place in a static environment such as a Petri or culture dish. However, there are disadvantages to seeding and culturing tissue in such an environment. For example, the lack of circulation of nutrients in these static systems results in a slow and ineffective seeding and culturing process. Moreover, cells which are seeded and cultured in a dynamic environment are more likely to tolerate the physiological conditions which exist in the human body once implanted. Thus, there exists a need for a dynamic environment in which to seed and culture tissue engineered vascular grafts and other prosthetic devices.

SUMMARY OF THE INVENTION

It is therefore an object of the invention to provide a dynamic environment for seeding, culturing, and testing vascular grafts, as well as other types of tissue grafts, of any desired length or diameter.

It is a further object of the invention to provide a precise mechanical device with a minimum of moving parts to provide such an environment.

It is yet a further object of the invention to provide a closed system free from contamination for sterilizing, seeding, culturing, storing, shipping, and testing tissue grafts.

In accordance with the present invention, there is provided an apparatus and method for sterilizing, seeding, culturing, storing, shipping, and testing vascular grafts and other prosthetic devices. In one specific embodiment of the invention, the apparatus comprises a fluid reservoir, a pump, at least one graft treatment chamber (treatment chamber), and an alternating pressure source. The apparatus includes a means for attaching at least one vascular graft directly in-line with the fluid reservoir. The pump forces fluid through the vascular graft, thereby subjecting it to radial and shear stresses. The alternating pressure source expands and contracts the treatment chamber, thus applying axial stress to the vascular graft secured within the treatment chamber during seeding and culturing. Applying shear, radial, and axial stresses to the vascular graft in this fashion during seeding and culturing simulates physiological conditions. This is believed to produce a prosthesis that is more likely to tolerate the physiological conditions found in the human body once implanted.

In another embodiment of the invention, the apparatus comprises a fluid reservoir, a pump, a cartilage treatment chamber, and an alternating pressure source. The pump forces fluid around and through the cartilage graft, while the alternating pressure source expands and contracts the treatment chamber, thereby applying alternating pressure to the cartilage graft secured within the treatment chamber during seeding and culturing.

In this manner, the invention advantageously utilizes a mechanically non-complex apparatus to create a dynamic environment in which to seed and culture tissue engineered vascular grafts or other implantable devices.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become more readily apparent from the following detailed description, which should be read in conjunction with the accompanying drawings in which:

FIG. 1 is a schematic diagram illustrating an apparatus according to the present invention for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis;

FIG. 2 illustrates a vascular graft treatment chamber containing a vascular graft; and

FIG. 3 illustrates a cartilage treatment chamber containing a cartilage graft.

DETAILED DESCRIPTION OF THE INVENTION

The following embodiments of the present invention will be described in the context of an apparatus and method for sterilizing, seeding, culturing, storing, shipping, and testing vascular and cartilage grafts, although those skilled in the art will recognize that the disclosed methods and structures are readily adaptable for broader application in for example, the fields of bone, tendon, and ligament grafts. Note that whenever the same reference numeral is repeated with respect to different figures, it refers to the corresponding structure in each such figure.

FIG. 1 discloses a system for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis. According to one preferred embodiment of the invention, this system primarily comprises a fluid reservoir 110, a pump 112, a treatment chamber 114, and an alternating pressure source 116.

Fluid reservoir 110 is used to store fluid for the system. Illustrative suitable reservoirs include a Gibco-BRL 1L media bag and any other rigid container capable of sterilization. Reservoir 110 may include a bacterial-retentive filter 111 so as to provide a direct source of gas to the fluid within the system. Examples of fluid which may be used in the system include, but are not limited to, sterilizing fluid, tanning fluid, fluid containing cells, or fluid containing a culture medium. It is to be understood that during testing, seeding, and culturing in a preferred embodiment, the fluid may be advantageously kept at human body temperature, and may be composed of a fluid which approximates the viscosity of human blood. One illustrative example of a solution which approximates the viscosity of blood is saline with glycerol.

The fluid contained in reservoir 110 is retrieved through fluid line 118 by pump 112. Fluid line 118, as well as all other fluid lines in the system, may be made of any type of medical grade, durable tubing -- such as silicone tubing -- which is suitable for transporting the fluid in use. Pump 112 may be any fluid pump which can achieve variable flow rates. One such pump is the Masterflex L/S Digital Drive peristaltic pump

manufactured by Cole-Palmer, although one skilled in the art could select from a variety of commercially available pumps. Pump 112 propels the fluid from reservoir 110 to treatment chamber 114 through fluid line 120. A pulse dampener 119 may be used along fluid line 120, between pump 112 and treatment chamber 114, to dampen the pulsatility of the fluid flow from pump 112. Suitable dampeners include the Masterflex airspring dampeners available from Cole-Palmer.

A timed valve 123 may also be utilized, in conjunction with a pressure control valve 148, to provide an alternating fluid pressure to treatment chamber 114. Specifically, when timed valve 123 is shut, pressure control valve 148 can be configured to create a desired pressure along fluid line 120. Once timed valve 123 is opened, this pressure is released through treatment chamber 114. By opening and closing timed valve 123 in this fashion, a controllable and repeatable pulsatile fluid flow may be provided to treatment chamber 114. This pulsatile flow in turn places a varying radial stress on vascular graft 126 (shown in FIG. 2) housed within treatment chamber 114, thereby producing a vascular graft that is more likely to tolerate the physiological conditions found in the human body once implanted. It is to be understood, however, that while in one embodiment of the invention, this pulsatile flow is created using timed valve 123, those of skill in the art will appreciate that there are other well-known means (including for example using the peristaltic pump without a flow dampener) for providing a pulsatile fluid flow to chamber 114.

A pressure transducer 140 and flow transducer 141 may also be placed in fluid line 120 between pump 112 and treatment chamber 114 to measure the pressure and flow of fluid into treatment chamber 114. Suitable pressure tranducers are the Transpac IV flow-through pressure gauge sold by Abbott Critical Care Systems, and the Honeywell flow-through pressure gauge sold by Newark Electronics. Signals from these transducers may be provided to a supervisory control and data acquisition system 149, which in one preferred embodiment, is the LabVIEW control and data acquisition system sold by National Instruments. As is discussed herein, system 149 is used to provide appropriate electronic signals to create, and then monitor, the physiological conditions in the system of FIG. 1 that are beneficial to the particular tissue being seeded and cultured.

FIG. 2 discloses a cross-sectional view of a treatment chamber 114. As shown in FIG.2, treatment chamber 114 comprises a main body 150 (made of, for example, cylindrical polycarbonate tubing), a top end cap 152, and a bottom end cap 154. Main body 150 is secured to cap 152 through any standard, leak-proof means, such as inner and outer threads or bonding agents. Main body 150 is secured to bottom cap 154 by a bellows 156, which provides a flexible joint in treatment chamber 114 along its vertical axis. Bellows 156 comprises any suitable elastomeric material, such as polyurethane or silicone, and may be dip molded. Bellows 156 is attached to body 150 and cap 154 by any suitable means, such as a band clamp.

Treatment chamber 114 additionally comprises a bottom mandrel 128 and a top mandrel 133. During construction of chamber 114, bottom mandrel 128 is inserted into treatment chamber 114 through the top of the chamber, and is then secured to bottom cap 150 through any appropriate means, such as the inner and outer threads shown in FIG. 2. The connection between cap 150 and mandrel 128 may be made leak proof through use of a silicone o-ring 155. As shown in FIG. 2 tubing 120 is inserted through the bore of mandrel 128 into treatment chamber 114, where it is securely attached to the vascular graft 126 by an adapter port 158. Adapter port 158 is firmly attached to mandrel 128 using, for example, inner and outer threads.

While adapter port 158 includes only one port in FIG. 2, a person of ordinary skill in the art will recognize that adapter ports 158 may be branched so that multiple ends of a branched (*e.g.*, y-shaped) vascular graft may be attached to adapter ports 158 in the manner described herein. Therefore, particular embodiments of the instant invention enable one skilled in the art to seed, culture, or treat single branch or multibranched vascular grafts.

Adapter port 158 is secured to vascular graft 126 by any conventional means, such as sutures, c-clips, surgical staples, medical grade bonding agents, tie wraps, or elastomeric bands. Alternatively, vascular graft 126 can be placed within a larger diameter port 158 and secured by compressing vascular graft 126 against port 158. Adapter port 158 may be comprised of a slightly porous material to allow for tissue ingrowth at the attachment sites.

Top cap 152, top mandrel 133, top port 158, and fluid line 122 are all attached in the same fashion as has been described in conjunction with the lower half of treatment chamber 114, once lower mandrel 128 is secured to treatment chamber 114.

In order to view vascular grafts within treatment chamber 14, a viewing port may be placed at any point on the chamber, or alternatively, the chamber may be made of an optically clear material such as polycarbonate or PVC. It is to be additionally understood that, while use polycarbonate material has been previously discussed, the components of treatment chamber 114 may be composed of any biocompatible, rigid material capable of being sterilized such as Teflon, PVC, or stainless steel. Moreover, it can also be made of a flexible material that will nevertheless assist in the control of fluid volume surrounding the vascular grafts during culture or cryopreservation.

Once treatment chamber 114 has been assembled, fluid may be passed through treatment chamber 114 beginning at bottom mandrel 128. More specifically, fluid enters mandrel 128 from fluid line 120 and then passes through vascular graft 126 to upper mandrel 133. By flowing fluid through the bore of vascular graft 126 in this fashion, shear stress is placed on the internal walls of the graft. In addition, if timed valve 123 and pressure control valve 148 are advantageously operated as described above (to create a pulsatile fluid flow), a varying radial stress is additionally placed on vascular graft 126. Applying shear and/or radial stresses to vascular graft 126 during seeding and culturing simulates physiological conditions found in the human body.

Once fluid flows through graft 126, it enters mandrel 133 and then exits through fluid line 125. As shown in FIG. 1, fluid line 125 connects mandrel 133 to inlet port 131, which allows the fluid to re-enter treatment chamber 114, and perfuse across the exterior surface of vascular graft 126. Once fluid has perfused across graft 126, it re-exits treatment chamber 114 through outlet port 130. Outlet port 130 is secured to fluid line 122.

In another embodiment of the invention, valve 129 is closed, for example, during seeding of graft 126. By closing valve 129, fluid is forced to enter vascular graft 126, perfuse through graft 126 due to back pressure, and then exit chamber 114 through port 130. Utilizing valve 129 in this fashion ensures that graft 126 is appropriately seeded

during the initial stages of treatment by forcing cells through graft 126. Once the appropriate level of seeding has been reached, valve 129 can be opened so that fluid flow proceeds in accordance with the above description.

Fluid passing through, and across, vascular graft 126 may cause the vascular graft to undulate. A support member, such as a skeletally-configured rod, may be attached by any conventional means to the adapter ports 158 to suppress such undulations. The vertical orientation of the grafts shown in FIG. 2 is not necessary if the graft is supported by such an internal support structure which does not unduly obstruct flow across the interior surfaces of the grafts which create shear stresses. Such support structures could additionally include a splint structure or rigid tubular screens with large, unobstructing openings.

As fluid is passing through treatment chamber 114, alternating pressure source 116 is used to repetitively elongate, and then contract, chamber 114 along its vertical axis. As previously mentioned, elongation of chamber 114 is enabled through use of bellows 156. In accordance with one embodiment of the invention, pressure source 116 comprises a pneumatic cylinder 170, a four-way solenoid valve 172, a flow control valve 174, a bottom position sensor 176, and a top position sensor 178. To create the alternativing vertical movement of chamber 114, air is provided to pressure source 116 from air supply 180. The air pressure is controlled by flow control valve 174, which is connected to four-way solenoid valve 172. Solenoid valve 172 is actuated by control system 149 in a fashion which creates a desired strain profile on the tissue to be seeded and cultured. More specifically, by actuating valve 172 using control system 149, the piston in pneumatic cylinder 170 is alternately driven between its top and bottom positions. Control system 149 receives electrical indications that these positions have been reached, respectively, by top position sensor 178 and bottom position sensor 176. By alternately elongating and then contracting chamber 114, a varying axial stress is advantageously placed on graft 126. This axial stress is advantageous as it simulates in-vivo conditions, resulting in three dimensional tissue that is more likely to display the biochemical, physical, and structural properties of native tissue.

One skilled in the art will understand that alternating pressure source 116 may be attached to either the top of bottom of treatment chamber 114 (which are separated by bellows 156) by any suitable means. One skilled in the art will also understand that the half of the treatment chamber which is not connected to alternating pressure source 116 should be secured in place so as to remain in a fixed position during treatment. Additionally, one skilled in the art will appreciate that the relationships between shear, radial and axial stresses imparted on a graft (including the frequency, magnitude and/or duration of each type of stress) may be varied as desired to optimize seeding and culturing conditions and/or to simulate physiological conditions *in vivo*.

While one specific embodiment of pressure source 116 has been described, any means for applying alternating pressure to chamber 114 along its vertical axis may be utilized. Such means would include, for example, a rotary motor driving an appropriate cam configuration.

As mentioned, treatment chamber 114 houses vascular graft scaffolding 126. As discussed in detail in both of the patents incorporated by reference below, graft 126 may illustratively consist of any knitted, braided, woven, felted, or synthesized materials that are bioresorbable and/or biocompatible, as well as any native graft scaffolding material. Treatment Chamber 114 may be made any length or diameter so as to hold a vascular graft scaffolding 126 of any length or diameter. This is advantageous, as the system may be used to sterilize, seed, culture, store, ship, and test vascular grafts of any size, such as coronary, carotid, iliac, and peripheral leg grafts.

However, while the system of FIG. 1 has been described in the context of a vascular graft scaffold 126, one skilled in the art will appreciate that the advantages of the present invention have much broader applicability. Thus, for example, as shown in FIG. 3, a treatment chamber 214, similar to treatment 114, may be devised to seed, culture and treat a cartilage graft 226 in conjunction with the system of FIG. 1. Chamber 214 is identical in many respects to chamber 114. However, chamber 214 includes only a single inlet 230 and a single outlet 231. Upper mandrel 200 and lower mandrel 202 are solid structures, and include no bore.

In accordance with this alternative embodiment of the present invention, fluid flows into inlet port 230 and then flows around the exterior of a nonporous plate 204, which is attached to upper adapter port 158. Fluid then perfuses through and across graft 226, through porous plate 206, and finally, through outlet port 231. While fluid is perfusing through and across graft 226 in this fashion, alternating pressure source 116 is used to alternatively expand and contract chamber 214. This expansion and contraction causes the volume between plates 204 and 206 to alternately compress and expand, thus placing a varying pressure on substrate 226.

In an alternative embodiment of the system of FIG. 3, plate 204 is a porous plate similar to porous plate 206. Thus, fluid entering chamber 214 through port 230 passes through plate 204. Graft 226 may attach to plate 204 or 206 as shown, or graft 226 may be mounted in a support structure (not shown) between plates 204 and 206. In a preferred method of use, fluid enters and exits chamber 214 through both ports 230 and 231. After cartilage graft substrate 226 is seeded and tissue matrix begins to grow, alternating pressure source 116 is used to alternatively expand and contract chamber 214 as described above. Expansion and contraction of chamber 214 causes the volume between plates 204 and 206 to alternately compress and expand, thus placing a varying pressure (contact pressure and/or fluid pressure) on substrate 226. For example, after cells are seeded under static flow conditions, the substrate 226 may be alternatingly relaxed and compressed between 0% strain and 75% during the early stages of growth, and between 25% and 75% during the later stages. One skilled in the art will appreciate that magnitude, frequency and duration of compression and relaxation of substrate 226 may be selected and varied to optimize culture conditions and/or to simulate *in vivo* conditions.

In yet another exemplary embodiment of the invention, the system of FIG. 1 contains a plurality of chambers 114 for treating a plurality of vascular grafts 126. Specifically, fluid line 120 may be split to connect to as many additional chamber inlets 121 as is desired, and fluid line 122 may be split to connect to as many additional chamber outlets 144 as is desired. While multiple pressure sources 116 may be utilized, one skilled in the art will appreciate that a single pressure source may be connected in parallel with multiple treatment chambers 114. Similarly, each treatment chamber 114

may be connected to a separate reservoir 110 and pump 112 so that multiple treatment chambers in a system would only share a single alternating pressure source 116. It is to be understood that pump 112 with multiple pump lines may also be used so that each treatment chamber 114 in the system would use the same alternating pressure source and same pump 112 (each using a different pump line), but would be connected to a different fluid reservoir 110. In this manner, a plurality of vascular grafts may be simultaneously seeded, cultured, or tested in accordance with the present invention.

The system disclosed in FIG. 1 may additionally include a pressure transducer 142 and flow transducer 143 at the outlet of treatment chamber 114. These transducers can provide additional data to control and data acquisition system 149, so that said system can ensure desirable strain profiles on the tissue to be cultured. In addition, a flow control valve 146 can be placed along fluid line 122, so that an ambient pressure within treatment chamber 114 that is greater than atmospheric pressure can be maintained if desired. Likewise, a pH indicator 161 and an oxygen content indicator 160 may be fluidly connected to reservoir 110, so as to periodically measure the pH level and oxygen content of the fluid in the system.

The inlet ports and outlet ports of treatment chamber 114 may be sealed in a known manner (e.g., luer locks or threaded plugs) so as to create a sealed treatment chamber free from contamination. The sealed chambers may be used to sterilize, store, and ship vascular grafts or other protheses. In particular, prior to placing a sealed chamber into the system of FIG. 1, vascular graft scaffolding 126 which is secured within sealed chamber 114 may be sterilized by some chemical means such as ethylene oxide or peracetic acid, radiation means such as an electron beam or gamma rays, or by steam sterilization. Sealed treatment chamber 114 containing the sterilized vascular graft scaffolding, or the sterilized vascular graft may then be placed back into the system FIG. 1 for seeding and culturing and unsealed without contaminating the system or the vascular graft.

Seeding and culturing of the vascular graft in treatment chamber 114 is generally accomplished by known techniques, with the added benefits and advantages gained from the radial, shear, and axial stresses placed upon the vascular graft during growth.

Examples of suitable seeding and culturing methods for the growth of three-dimensional cell cultures are disclosed in U.S. Patent No. 5,266,480 and in U.S. patent application serial no. 08/487,749, which was filed June 7, 1995 and is entitled "In Vitro Preparation of Tubular Tissue Structures by Stromal Cell Culture on a Three Dimensional Framework", both of which are incorporated herein by reference. The techniques described in U.S. Patent No. 5,266,480 and in U.S. patent application serial no. 08/487,749 for establishing a three-dimensional matrix, inoculating the matrix with the desired cells, and maintaining the culture may also be readily adapted by a person of ordinary skill in the art for use with the present invention.

Once the vascular graft has reached the desired level of cell density, a preservative may then be pumped into treatment chamber 114. Once the treatment chamber is filled with the preservative, the inlet ports and outlet ports of the chamber may be closed, again creating a sealed chamber which may then be used to store and/or ship the cultured and preserved vascular graft. Preferably, the preservative is a cryo-preservative so that the graft may be frozen in chamber 114. In this manner, sealed treatment chamber 114 may be used to sterilize, culture, store, and ship vascular grafts or other protheses.

Various embodiments of the invention have been described. The descriptions are intended to be illustrative, not limitative. Thus, it will be apparent to those skilled in the art that modifications may be made to the invention as described without departing from the scope of the claims set out below.

We claim:

1. An apparatus, comprising:

a vascular graft designed to facilitate three-dimensional tissue growth on said graft, said graft comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a chamber having a first port and a second port for flow of fluid therethrough;

means for connecting said vascular graft within said chamber;

means for imparting radial and shear stresses to said vascular graft; and

means for applying axial stresses to said vascular graft.

2. The apparatus of claim 1, wherein said first port and said second port are fluidly connected within said chamber by the bore of said vascular graft, and wherein said imparting means comprises a means for forcing fluid from said first port to said second port through said vascular graft such that fluid comes into contact with said vascular graft.

3. The apparatus of claim 2, wherein said imparting means further comprises a means for creating an alternating pressure within said vascular graft.

4. The apparatus of claim 3, wherein said chamber further comprises a third port and a fourth port for flow of fluid therethrough, and wherein said third and fourth ports are fluidly connected by said chamber such that fluid from said third port to said forth port comes in contact with the exterior surfaces of said vascular graft.

5. The apparatus of claim 3, wherein said means for creating pressure comprises a means for alternating the pressure of the fluid flow into said vascular graft.

6. The apparatus of claim 5, wherein said means for alternating the pressure of the fluid flow comprises a timed valve.

7. The apparatus of claim 1, wherein said applying means comprises a means for elongating said chamber.

8. The apparatus of claim 7, wherein said chamber includes an expandable bellows, and said elongating means comprises a pneumatic piston.

9. The apparatus of claim 8, further comprising a pump for providing an alternating flow of fluid to said chamber through said first port.

10. An apparatus for seeding and culturing vascular grafts, comprising:

a chamber defined by top and bottom walls and at least one side wall;

a first and a second fluid inlet to said chamber and a first and a second fluid outlet from said chamber;

a vascular graft designed to facilitate three-dimensional tissue growth on said graft, said graft comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a first fitting mounted within the chamber, in fluid communication with the first fluid inlet, said fitting being configured and dimensioned to receive and hold open a first end of said vascular graft to permit flow of fluid through said graft;

a second fitting mounted within the chamber, in fluid communication with the first fluid outlet, said fitting being configured and dimensioned to receive and hold open a second end of said vascular graft to permit flow of fluid from said graft;

a fluid supply system communicating with the first fluid inlet to provide pulsatile fluid flow through said first fitting and communicating with said second fluid inlet to provide fluid flow across the exterior of said vascular graft housed within said chamber and through said second fluid outlet, said second fluid inlet and said second fluid outlet in fluid communication through said chamber; and

a means for expanding said chamber to apply axial stresses to said vascular graft.

11. The apparatus according to claim 10, wherein said at least one fitting is mounted on the top wall of the chamber such that a vascular graft received thereon hangs downward in a generally vertical orientation.

12. The apparatus according to claim 11, wherein said outlets communicate with the fluid supply system to provide a closed fluid supply system.

13. A method for seeding and culturing a vascular graft, comprising:

providing a vascular graft designed to facilitate three-dimensional tissue growth on said graft, said graft comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

exposing said vascular graft to a fluid media;

imparting radial and shear stresses to said vascular graft during at least one of said seeding or culturing to simulate physiological conditions; and

applying axial stresses to said vascular graft during said at least one of said seeding or culturing to simulate physiological conditions.

14. The method of claim 13, wherein:

said exposing step comprises, directing a fluid flow through said vascular graft; and

said imparting step comprises, forcing said fluid media through said vascular graft with a pulsatile flow such that radial and shear stresses are imparted to said vascular graft.

15. An apparatus, comprising:

a cartilage graft designed to facilitate three-dimensional growth on said graft, said graft comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a chamber having a first port and a second port for flow of fluid therethrough;

means for connecting said cartilage graft within said chamber;

means for imparting shear stresses to said cartilage graft; and

means for applying compressive forces to said cartilage graft.

16. The apparatus of claim 15, wherein said chamber has a variable length and said applying means comprises a means for changing the length of said chamber.

17. The apparatus of claim 16, wherein said applying means further comprises a first opposing plate and a second opposing plate within the chamber, said cartilage graft being disposed between the plates such that decreases in the length of the chamber decrease a distance between the plates and apply compressive forces to said cartilage graft.

18. The apparatus of claim 17, wherein said chamber further includes an expandable bellows, and said means for changing length comprises a pneumatic piston.

19. An apparatus, comprising:

a graft designed to facilitate three-dimensional tissue growth on said graft, said graft comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a chamber having a first port and a second port for flow of fluid therethrough;

means for connecting said graft within said chamber;

means for imparting shear stresses to said graft; and

means for applying axial stresses to said graft.

20. The apparatus of claim 19, wherein said graft is a vascular graft having a lumen, and said apparatus further comprises a means for varying pressure within the lumen thereby imparting radial stresses to said graft.

21. The apparatus of claim 19 wherein said graft is any of a vascular graft, a tendon graft, a ligament graft or a bone graft.

22. The apparatus of claim 21, wherein said applying means comprises a means for elongating said chamber.

23. The apparatus of claim 22, wherein said chamber includes an expandable bellows, and said elongating means comprises a pneumatic piston.

1/2

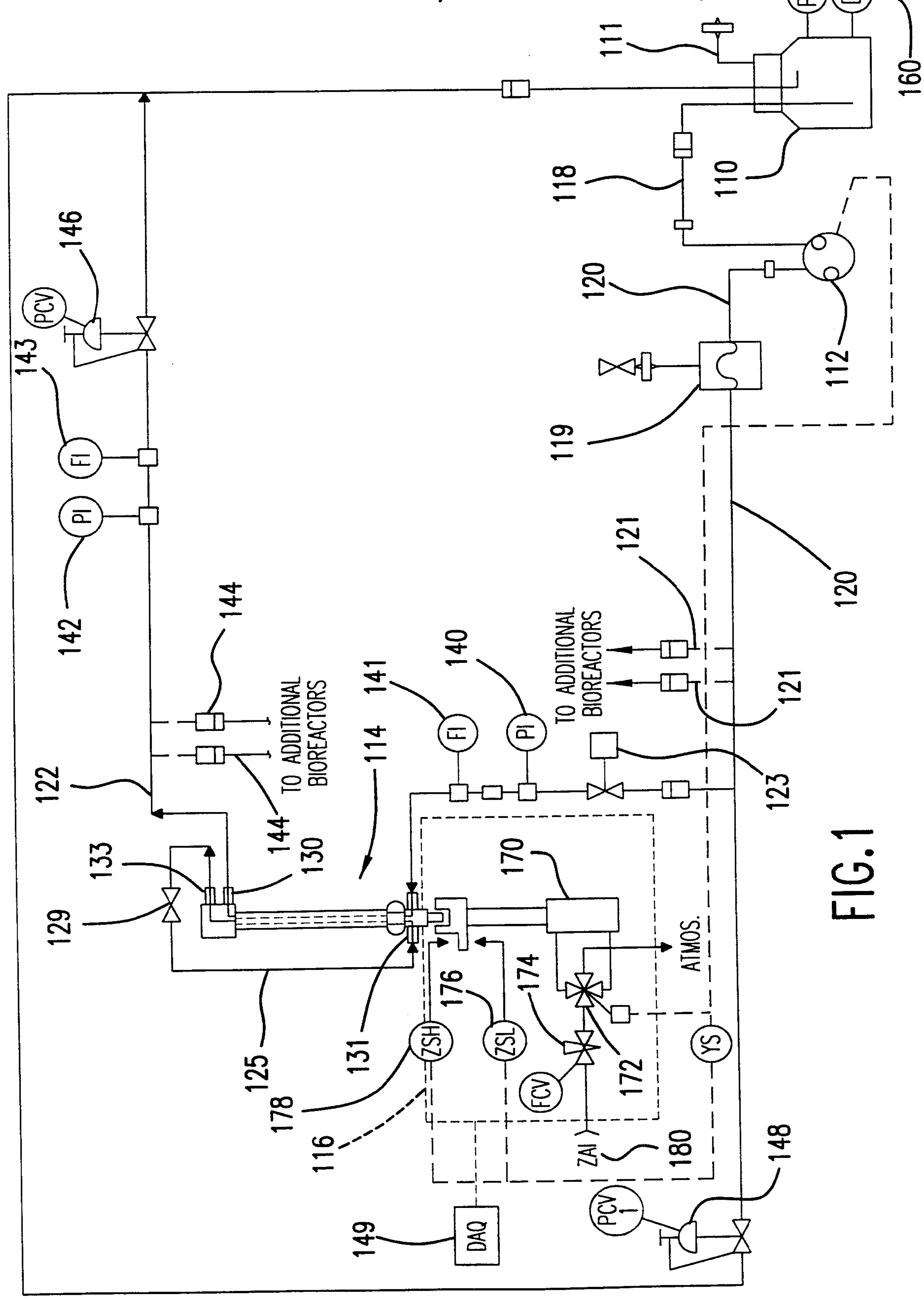

PH
DO
PCV
FI
PI
TO ADDITIONAL BIOREACTORS
TO ADDITIONAL BIOREACTORS
ATMOS.
ZSH
ZSL
FCV
YS
ZAI
DAQ
PCV 1
110
111
112
114
116
118
119
120
121
122
123
125
129
130
131
133
140
141
142
143
144
146
148
149
160
161
170
172
174
176
178
180

FIG.1

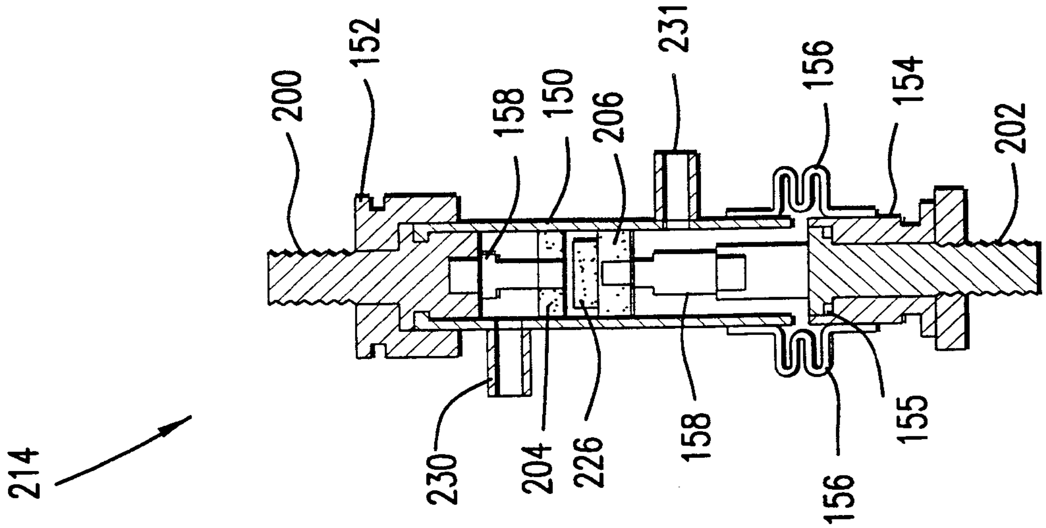

214
200
152
158
150
206
231
156
154
202
230
204
226
158
156
155

FIG.3

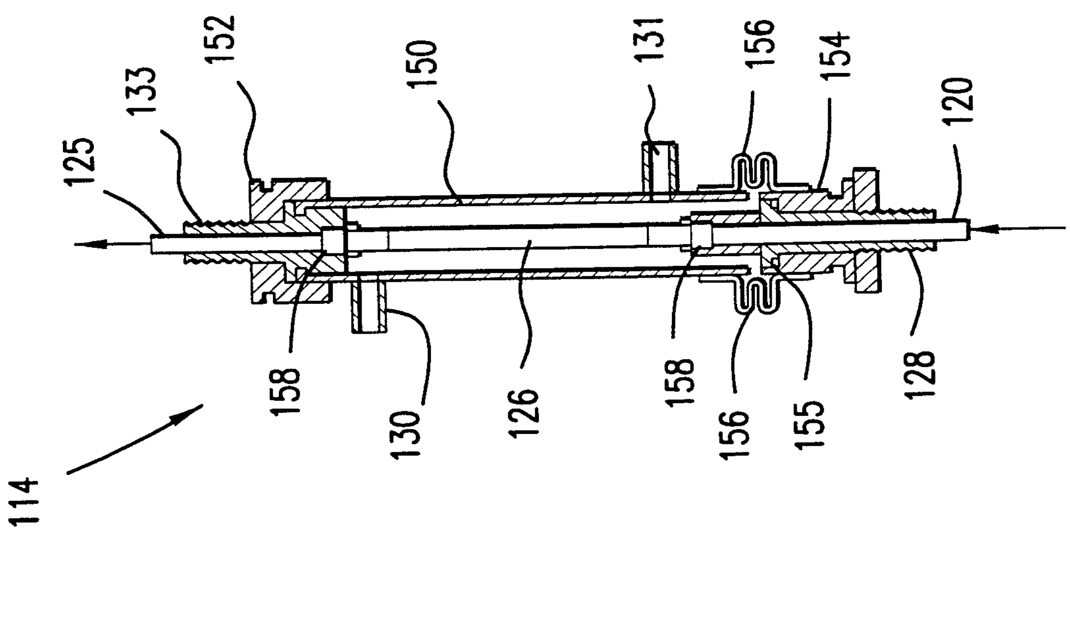

114
125
133
152
150
131
156
154
120
158
130
126
158
156
155
128

FIG.2

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/01003

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61F2/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 49799 A (ADVANCED TISSUE SCIENCES, INC.) 31 December 1997 (1997-12-31)	1-3,5,6, 13-15, 19-21
A	the whole document ---	10
Y	WO 92 14419 A (BAXTER INTERNATIONAL INC.) 3 September 1992 (1992-09-03) page 17, line 1 -page 18, line 18 ---	1-3,5,6, 13-15, 19-21
A	WO 93 01843 A (UNIVERSITY OF LEICESTER) 4 February 1993 (1993-02-04) -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2000	19/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Smith, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/01003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9749799 A	31-12-1997	US 5792603 A	11-08-1998
		AU 3581797 A	14-01-1998
		CA 2261027 A	31-12-1997
		EP 0927245 A	07-07-1999
WO 9214419 A	03-09-1992	CA 2101557 A	15-08-1992
		DE 69210225 D	30-05-1996
		DE 69210225 T	05-12-1996
		EP 0571527 A	01-12-1993
		JP 6505187 T	16-06-1994
		US 5376110 A	27-12-1994
WO 9301843 A	04-02-1993	AU 2361792 A	23-02-1993